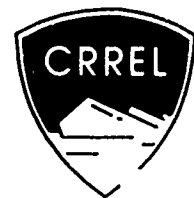


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# Evaluation of Pre-extraction Analytical Holding Times for Nitroaromatic and Nitramine Explosives in Water

Clarence L. Grant, Thomas F. Jenkins and Susan M. Golden August 1993

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### **Abstract**

A study was conducted to experimentally evaluate the maximum acceptable pre-extraction analytical holding times (MHTs) for three nitroaromatic compounds and two nitramines in water. Three fortified waters (reagent-grade water, ground water and surface water) were utilized in the study. Analytes investigated were HMX, RDX, TNB, TNT and 2,4-DNT, all at the low  $\mu\text{g/L}$  level. Subsamples of each water sample were held for periods of 0, 3, 7, 14, 28 and 70 days at either room temperature ( $22^\circ\text{C}$ ) or under refrigeration ( $2^\circ\text{C}$ ). Samples were analyzed by RP-HPLC without preconcentration.

The two nitramines, HMX and RDX, were stable over the entire period for all waters under both storage temperatures. For the three nitroaromatics (TNB, TNT and 2,4-DNT) the results were very different. For the surface water, significant losses of TNB and TNT occurred in only a day or two, even under refrigeration. Loss of these analytes was accompanied by an increase in the concentration of their microbiological degradation products 3,5-dinitroaniline and 4-amino-2,6-dinitrotoluene, respectively. The rate of loss of 2,4-DNT was much slower in the surface water than either for TNB or TNT. The rates of loss of the three nitroaromatics were much slower in the reagent-grade water and ground water than in the surface water. The recommended MHT for nitroaromatics and nitramines in relatively sterile water samples is 50 days under refrigeration. Surface water samples, or samples likely to have significant microbial activity, should be preserved in the field prior to shipment or analyzed on-site, or the loss of TNB and TNT is likely to be significant. Concern is voiced over the use of organic solvents for fortification in holding time studies because of their potential for enhancement or suppression of microbiological degradation rates.

For conversion of SI metric units to U.S./British customary units of measurement consult ASTM Standard E380-89a, *Standard Practice for Use of the International System of Units*, published by the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103.



**U.S. Army Corps  
of Engineers**  
Cold Regions Research &  
Engineering Laboratory

## **Evaluation of Pre-extraction Analytical Holding Times for Nitroaromatic and Nitramine Explosives in Water**

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August 1993

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## PREFACE

This report was prepared by Dr. Clarence L. Grant, Professor Emeritus, University of New Hampshire; Dr. Thomas F. Jenkins, Research Chemist, Geological Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory (CRREL); and Susan M. Golden, Science and Technology Corporation. Funding was provided by the U.S. Army Environmental Center (formerly the U.S. Army Toxic and Hazardous Materials Agency), Aberdeen Proving Ground, Maryland, Martin H. Stutz, Project Monitor.

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# Evaluation of Pre-extraction Analytical Holding Times for Nitroaromatic and Nitramine Explosives In Water

CLARENCE L. GRANT, THOMAS F. JENKINS AND SUSAN M. GOLDEN

## INTRODUCTION

The U.S. Environmental Protection Agency has issued a draft reversed-phase high-performance liquid chromatographic (RP-HPLC) method for the determination of nitroaromatic and nitramine explosives in water (EPA 1992). The method was based on research results obtained at the U.S. Army Cold Regions Research and Engineering Laboratory (Jenkins et al. 1986, 1992, Miyares and Jenkins 1990, 1991). A maximum pre-extraction sample holding time (MHT) was specified to be the same as for semivolatile organics (7 days). To our knowledge, this MHT was based on best judgment rather than on experimental data.

In a recent report, MHTs were estimated for nitroaromatics and nitramines in water (Maskarinec et al. 1991). Distilled water, ground water and surface water samples were fortified with stock solutions of 1,3,5,7-octahydro-1,3,5,7-tetranitrotetrazocine (HMX), 1,3,5-hexahydro-1,3,5-trinitrotriazine (RDX), 2,4,6-trinitrotoluene (TNT) and 2,4-dinitrotoluene (2,4-DNT) in acetonitrile (ACN) and stored for periods up to a year at 4° and 25°C. Quadruplicate subsamples were analyzed for each combination of water type and storage temperature at each of eight time periods, and the resulting concentrations of each analyte were plotted as a function of holding time. MHTs were estimated using two procedures: a modified version of an ASTM (1986) procedure and one reported by Prentice et al. (1986). A discussion of these procedures and a description of an alternative modification of the ASTM method are available elsewhere (Grant et al. 1993). Maskarinec et al. (1991) reported conservative MHTs because the estimates depended on factors such as water source and analyte concentration and because there was considerable scatter in the data. Although

HMX and RDX were reasonably stable under refrigeration for 30-50 days, they stated that low concentrations of TNT and DNT must be determined "very quickly."

A concern with the Maskarinec et al. (1991) study is the addition of an organic solvent (ACN) during analyte fortification. In particular, addition of 10% ACN has been recommended as one element in a method of preservation to retard chemical and biological changes in water samples containing nitroaromatics and nitramines (Miller et al. 1983). However, current standard procedures do not specify the addition of any preservative. While the concentration of ACN used by Maskarinec et al. in their fortified waters was not reported, it was probably considerably less than the 10% recommended by Miller et al. for preservation. Nevertheless, the concentration of ACN was undoubtedly many orders of magnitude greater than that of the analytes, and the extent of either reduction in microbial activity due to toxicity to microorganisms or stimulation of activity due to the availability of a readily degradable source of organic carbon is uncertain. Hence, we feel that MHTs developed in the absence of ACN may be more representative of actual sample behavior than those reported by Maskarinec et al.

## OBJECTIVE

The objective of the following study was to re-examine the issue of MHTs for nitroaromatic and nitramine explosives in water without the use of organic solvents during analyte fortification. MHTs were obtained by plotting the concentration of analytes as a function of holding time and using a modified version of the statistical procedure recommended by the ASTM (Grant et al. 1993). We

used the same four analytes studied by Maskarinec et al. (1991) along with 1,3,5-trinitrobenzene (TNB), a commonly occurring phototransformation product of TNT (Burlinson 1980, Spanggord et al. 1980). Microbiological degradation of TNB and TNT were verified, not only by their disappearance, but also by the appearance of selected transformation products: 3,5-dinitroaniline (3,5-DNA) from TNB, and 2-amino-4,6-dinitrotoluene (2-AmDNT) and 4-amino-2,6-dinitrotoluene (4-AmDNT) from TNT (Won et al. 1974, McCormick et al. 1976, Goerlitz and Franks 1989).

## EXPERIMENTAL METHODS

### Chemicals

All standards and test solutions were prepared from Standard Analytical Reference Materials (SARMs) obtained from the U.S. Army Environmental Center (formerly the Toxic and Hazardous Materials Agency), Aberdeen Proving Ground, Maryland. Aqueous standards and test solutions were prepared in reagent-grade water obtained from a Milli-Q Type I reagent-grade water system. The methanol used in preparing the analytical standards and the HPLC eluent was HPLC-grade from Alltech. The HPLC eluent was prepared by combining equal volumes of methanol and water and vacuum filtering through a 0.45- $\mu$ m nylon membrane to degas it and remove particulate matter.

### Analyte spiking solutions

All analyte spiking solutions were prepared in water. SARMs for TNT, 2,4-DNT, TNB, RDX and HMX were placed in individual brown glass jugs, reagent-grade water was added, and the contents were stirred at room temperature for a week. The solutions were then filtered through 0.45- $\mu$ m nylon membranes into clean, brown glass jugs. No solvents, other than water, were used in the preparation of these solutions.

The concentration of analyte in each aqueous spike solution was determined against standards prepared in methanol or acetonitrile (Jenkins et al. 1986, EPA 1992) diluted 1:1 with reagent-grade water prior to analysis. A multianalyte spiking solution was prepared by combining appropriate volumes of these individual analyte solutions and filtering through a 0.45- $\mu$ m nylon membrane. The combined analyte spike solution was stored in a brown glass bottle in the refrigerator until used.

### Water samples

Blank water samples were collected from three sources: reagent-grade water from a Milli-Q Type I water system, surface water from the Connecticut River in West Lebanon, N.H., and ground water from a deep well in Enfield, N.H. (Table 1).

### Analyte spiking

The three blank water samples were fortified by combining 20 mL of the filtered multianalyte spike solution with 20 mL of filtered aqueous HMX solution in each of three separate 2-L volumetric flasks. The need to add additional HMX solution was due to its limited solubility. Spike solutions were diluted to volume with blank water from the appropriate source and mixed thoroughly. Subsamples were prepared by filling test vials to overflowing and immediately capping with Teflon-coated caps. The day 0 vials were set aside, and the remaining vials were divided into two sets. One set was stored in the dark at room temperature, and the other was stored in the dark under refrigeration.

### Test parameters

For all water samples, two storage conditions were used (Table 2): room temperature ( $22 \pm 2^\circ\text{C}$ ) and refrigerator ( $2 \pm 2^\circ\text{C}$ ). Subsamples stored under these conditions were analyzed after 0, 3, 7, 14, 28 and 70 days of storage. Triplicate portions were analyzed for each storage temperature and time, with the exception of day 0, on which six replicates

Table 1. Chemical properties of water samples.

Property	Reagent-grade water	Surface water	Ground water
pH	6.4	5.5	5.5
Conductivity ( $\mu\text{mhos/cm}$ )	1	99	198
Dissolved oxygen (mg/L)	4.6	4.6	4.8



**Table 2. Experimental factors for water holding time study.**

<i>Factors</i>	<i>No. of levels</i>	<i>Levels</i>
Analytes	5	HMX, RDX, TNB, TNT, 2,4-DNT
Waters	3	Reagent Type I, Surface water, Ground water
Storage temperature (°C)	2	22 ± 2, 2 ± 2
Storage time (days)	6	0, 3, 7, 14, 28, 70
Replicates	3-6	6 for day 0, 3 for all other days

were used to establish initial concentrations. Prior to HPLC analysis, each sample was brought to room temperature, and 5.00 mL was combined with 5.00 mL of methanol. The combined sample was mixed and filtered through a 0.5- $\mu$ m MillexSR filter.

#### RP-HPLC analysis

All water samples were analyzed by RP-HPLC. Analysis was conducted on a modular system composed of a Spectra-Physics Model SP8800 ternary HPLC pump, a Spectra-Physics Spectra 100 UV variable-wavelength detector set at 254 nm (cell path 1 cm), a Dynatech Model LC 241 autosampler equipped with a Rheodyne Model 7125 Sample Loop Injector, a Hewlett Packard 3393A digital integrator and a linear strip-chart recorder.

All samples were analyzed on a 25-cm  $\times$  4.6-mm (5 $\mu$ ) LC-18 column (Supelco) eluted with 1:1 methanol/water (v/v) at 1.5 mL/min (Jenkins et al. 1988). Samples were introduced by overfilling a 100-mL sampling loop. Retention times of the analytes of interest are shown in Table 3. Confirmation of identities of analytes and transformation products were obtained on a 25-cm  $\times$  4.6-mm (5 $\mu$ ) LC-CN column (Supelco) under the same operational conditions (Table 3).

Concentration estimates were obtained for most analytes from peak heights from the digital integrator. Because of problems with the baseline, particularly for the surface water samples (Fig. 1), results for HMX were obtained by manual peak height measurement. Manual peak heights were also used to estimate the very small peaks for the transformation products throughout (Fig. 2) and for TNB in the surface water after 70 days of storage at room temperature (Fig. 3).

#### Data analysis

The means and standard deviations were calculated for each of 170 sets of triplicate measurements and 15 sets of 6 replicates. Suspect individual measurements were flagged on the basis of extreme values of the % RSD (> 50%) and inconsis-

tencies in the overall pattern for that compound. Each suspect value was checked for possible computation or transcription errors. Nine individual extreme values (three for HMX, one for RDX, two for TNT, one for TNB, one for 3,5-DNA and one for 4-AmDNT) with no assignable cause were arbitrarily excluded because they produced large distortions of both means and standard deviations. Exclusion of more than one datum from a triplicate set occurred only once. These nine exclusions, which all occurred in the surface water samples, amounted to less than 1.5% of the values.

A modified version of the ASTM procedure was used to estimate MHTs where appropriate. Except for day 0, triplicate measurements were used throughout. To gain degrees of freedom (d.f.) and to fairly represent precision for the entire experiment, pooled standard deviations were calculated for the five sets of triplicates and one set of six replicates for each water-storage condition where rapid degradation was absent. This produced more d.f. for the standard deviation estimate than the nine that would have been obtained if we had run ten replicates on day 0 as suggested

**Table 3. Retention times of test analytes and transformation products for two reversed-phase columns.**

<i>Analyte</i>	<i>Retention time (min)</i>	
	<i>LC-18*</i>	<i>LC-CN†</i>
HMX	2.6	11.7
RDX	3.8	8.1
TNB	4.9	7.3
3,5-DNA	6.8	8.0
TNT	7.8	10.4
4-AmDNT	8.7	9.4
2-AmDNT	9.0	9.4
2,4-DNT	9.4	8.5

\* Eluent 1:1 methanol/water at 1.5 mL/min.

† Eluent 65:23:12 water/acetonitrile/methanol at 1.2 mL/min.

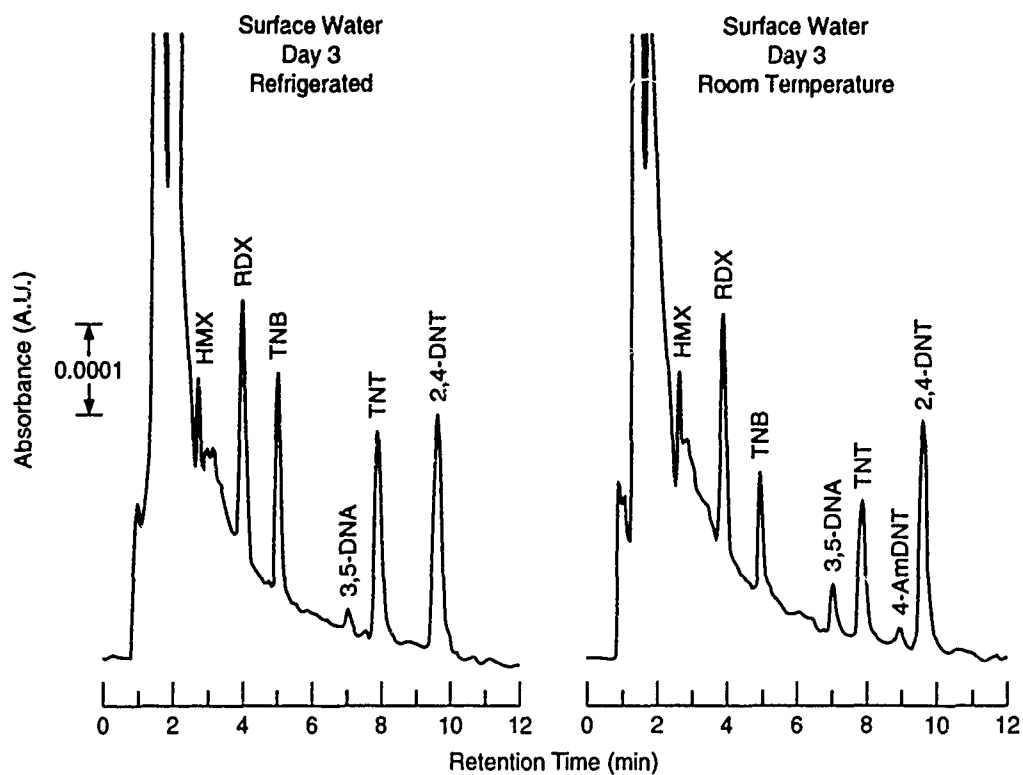


Figure 1. Chromatograms of surface water showing the large sloping background.

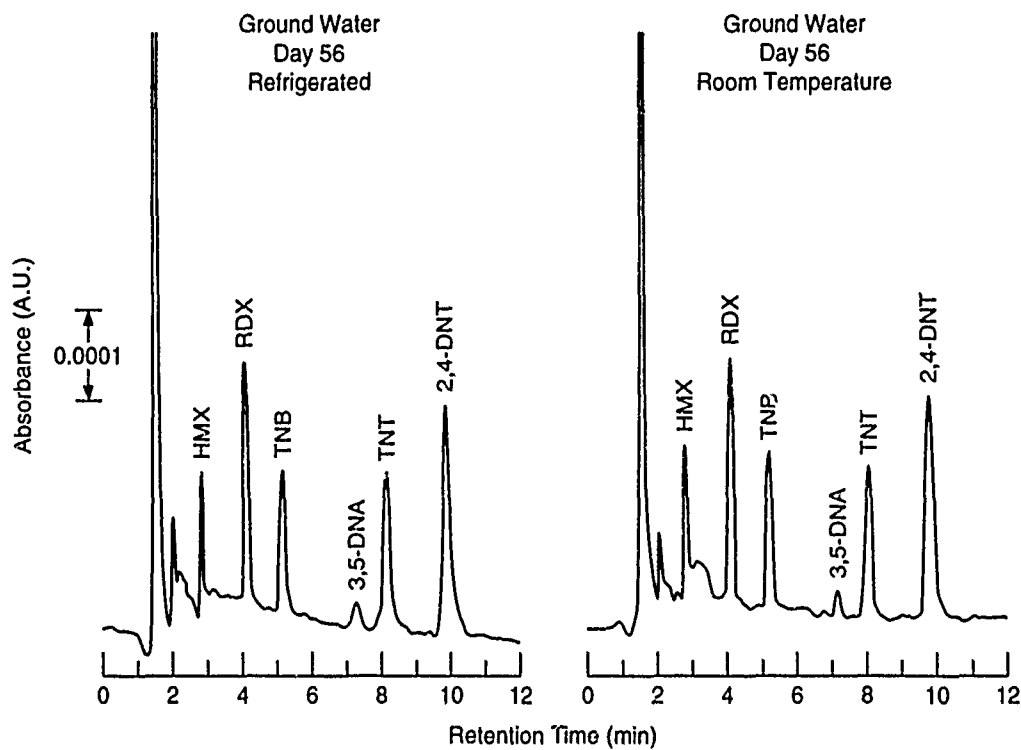


Figure 2. Chromatograms of ground water showing small 3,5-DNA peaks.

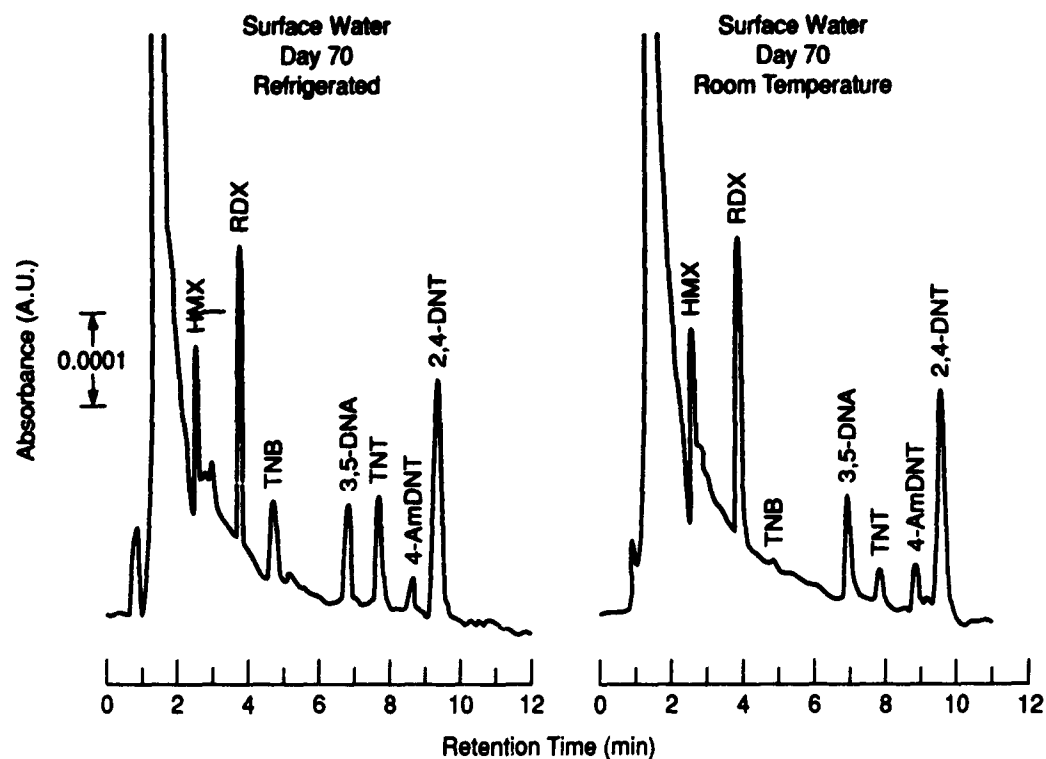


Figure 3. Chromatograms of surface water showing very small TNB peaks after 70 days of storage at room temperature.

by ASTM. Confidence intervals (99%) were calculated using the pooled standard deviation with  $n = 3$ . These confidence limits were centered on day 0 means and examined for the time when they intersect the best graphical fit to a plot of mean concentration vs. time. This procedure worked well, and the results should be very comparable to the standard ASTM procedure.

## RESULTS AND DISCUSSION

### Overview

Mean analyte concentrations and percent relative standard deviations (RSD) for the three fortified water samples as determined by the analysis of six replicates on day 0 are given in Table 4. The mean concentration of HMX in the surface water was significantly ( $\alpha = 0.05$ ) lower than in the other two waters. All other comparisons of day 0 mean concentrations in the three waters showed very minor variations, and the only other significant difference ( $\alpha = 0.05$ ) was the slightly low mean for TNB in the surface water. The precision of the measurements was excellent, with an average percent RSD of 2.4%, and this provided the ability to

detect very small differences in means. We believe that the only difference of practical significance is the low mean for HMX. Unfortunately the cause of this low value is currently unknown with certainty, but a large sloping background under the HMX peak in the surface water sample offers a plausible explanation, since this background is absent in the other samples.

The excellent precision observed for day 0 data continued throughout the study. Therefore, the 99% confidence limits computed according to our modified ASTM procedure showed only very small departures from day 0 means. Percent differences between day 0 means and 99% confidence intervals are summarized in Table 5. The ASTM procedure requires these differences not to exceed  $\pm 15\%$ . Since all of these values are much less than  $\pm 15\%$ , very small changes from day 0 concentrations can lead to estimates of quite short MHTs. In some cases these times may be shorter than necessary, since sampling variations are often  $\pm 15\%$  or more within dynamic hydrologic systems. It is also interesting to note that the refrigerated samples often show poorer precision than the samples stored at room temperature (Table 6). Not surprisingly the surface water shows poorer precision

**Table 4. Initial concentrations of nitroaromatics and nitramines in fortified waters estimated by RP-HPLC.**

Compound	Reagent-grade water		Surface water		Ground water	
	$\bar{X}$ ( $\mu\text{g/L}$ )	RSD (%)	$\bar{X}$ ( $\mu\text{g/L}$ )	RSD (%)	$\bar{X}$ ( $\mu\text{g/L}$ )	RSD (%)
HMX	46.0	2.1	38.5*	4.6	47.5	3.4
RDX	72.6	1.4	72.0	1.7	73.2	2.9
TNB	49.2	0.9	47.2*	3.8	49.0	1.3
TNT	50.6	1.2	51.1	4.9	51.1	1.2
2,4-DNT	41.4	1.4	41.2	2.0	41.5	2.9

\* Means are significantly different ( $\alpha = 0.05$ ) from the means for those analytes in reagent-grade water and ground water.

than the ground water and reagent water samples.

#### Behavior of analytes as a function of holding time

The mean concentrations of the five fortified analytes and three transformation products are presented in Table 6 as a function of holding time and storage condition for the reagent-grade water, surface water and ground water, respectively. Since the nitroaromatics show much less stability than the nitramines, we will discuss the two groups separately.

#### Nitramines

The mean concentrations of HMX and RDX are plotted as a function of holding time in Figures 4 and 5, respectively. For each of the three water samples, a single line was drawn through the points for both storage temperatures because there was no significant effect of temperature in any case.

Because of the outstanding within-day precision of measurements, the plots offer strong evidence that there was a small but significant day-to-

day calibration error. Consistently low values for both HMX and RDX on days 3 and 7 caused deviations that are logically assigned to calibration bias. Since standards were prepared fresh on each day of analysis, such an error was certainly possible. Clearly HMX and RDX did not rapidly degrade and then re-form!

By ignoring the 3- and 7-day points, reasonable MHTs could be estimated for HMX by the modified ASTM procedure. From Figure 4 we see that these times range from 40 to 50 days. Since the mean difference between the 99% confidence limits and the day 0 values was only 5.7% for HMX, these MHT estimates seem quite conservative. They also agree with the estimates reported by Maskarinec et al. (1991) for refrigerated water.

If the ASTM procedure had been rigorously applied to the RDX results (Fig. 5), we would have concluded that MHTs were only about one day, because the 99% confidence limits all lie between 69.2 and 70.8  $\mu\text{g/L}$ . This conclusion would result from the very small difference (3.8%) between day 0 means and the 99% confidence limits and the large difference in day 0 and day 3 means. In

**Table 5. Percent change of day 0 means required to reach the lower 99% confidence intervals for MHT estimation.**

Analyte	Reagent-grade water		Surface water		Ground water		Mean (%)
	22°C (%)	2°C (%)	22°C (%)	2°C (%)	22°C (%)	2°C (%)	
HMX	3.63	4.16	6.97	7.70	5.51	6.36	5.72
RDX	3.16	3.42	3.89	4.13	3.22	4.91	3.79
TNB	5.66	6.47	8.56	7.04	4.46	4.16	6.06
TNT	2.85	5.08	6.41	9.99	3.56	4.84	5.46
2,4-DNT	3.11	5.18	4.13	3.14	4.01	5.22	4.13
Column mean	3.68	4.86	5.99	8.92	4.15	5.10	5.03
Water mean		4.27		6.20		4.63	

**Table 6. Concentrations of analytes and transformation products as a function of holding time and storage condition.**

		Concentration (µg/L)											
		0 days		3 days		7 days		14 days		28 days		70 days	
Compound	Storage	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
Reagent-grade water													
HMX	Room temp.	45.97	0.97	44.97	0.12	45.97	1.27	46.23	0.92	46.57	0.49	40.63	1.42
	Refrigerator	45.97	0.97	45.90	0.85	45.80	0.40	46.70	1.01	46.47	0.21	39.70	2.23
RDX	Room temp.	72.56	1.03	65.74	0.84	65.97	1.29	67.00	1.47	67.91	1.19	64.14	2.17
	Refrigerator	72.56	1.03	65.64	0.36	67.77	0.88	68.00	0.50	66.65	0.48	64.73	3.40
TNB	Room temp.	49.21	0.44	49.31	0.36	48.08	0.63	43.99	1.02	42.13	2.11	37.02	3.63
	Refrigerator	49.21	0.44	49.80	0.19	49.45	0.55	48.92	1.02	50.94	0.30	45.69	4.88
TNT	Room temp.	50.56	0.62	50.62	0.66	50.86	0.62	49.20	0.80	48.13	0.40	42.31	1.65
	Refrigerator	50.56	0.62	51.06	0.78	51.27	0.75	51.62	0.52	51.45	0.05	47.24	3.79
2,4-DNT	Room temp.	41.35	0.59	40.40	0.06	41.83	0.43	41.57	0.90	41.43	1.06	36.94	1.09
	Refrigerator	41.35	0.59	41.38	0.50	41.44	1.26	42.08	0.55	41.64	0.55	38.97	2.89
4-AmDNT	Room temp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Refrigerator	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,5-DNA	Room temp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.47	0.44
	Refrigerator	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Surface water													
HMX	Room temp.	38.50	1.76	34.30	0.56	27.07	2.50	39.13	1.10	36.33	1.65	32.23	0.23
	Refrigerator	38.50	1.76	28.43	1.62	29.20	1.57			36.27	1.80	32.70	1.51
RDX	Room temp.	71.96	1.70	64.12	1.13	65.84	0.73	69.06	2.40	67.96	1.00	66.89	1.93
	Refrigerator	71.96	1.70	66.00	2.37	67.01	0.20	67.68	2.16	68.06	2.03	63.81	0.52
TNB	Room temp.	47.24	1.81	15.37	2.48	7.63	1.01	4.51	0.85	3.54	3.18	3.50	3.91
	Refrigerator	47.24	1.81	32.77	1.41	20.69	1.09	10.63	0.93	4.94	0.66	5.24	3.62
TNT	Room temp.	51.09	2.52	30.47	1.50	25.96	1.35	21.84	1.10	14.68	0.46	6.52	1.89
	Refrigerator	51.09	2.52	43.26	0.63	36.40	3.12	28.38	1.19	22.91	0.09	14.06	5.73
2,4-DNT	Room temp.	41.20	0.82	39.53	1.47	41.02	0.88	39.24	0.76	38.95	0.82	34.37	1.21
	Refrigerator	41.20	0.82	41.24	0.75	40.58	0.37	40.36	0.54	40.14	1.02	35.04	0.72
4-AmDNT	Room temp.	0.00	0.00	0.00	0.00	0.00	0.00	7.24	0.93	8.31	3.26	12.09	0.65
	Refrigerator	0.00	0.00	0.00	0.00	0.00	0.00	5.13	0.68	7.27	0.79	7.45	0.83
3,5-DNA	Room temp.	0.00	0.00	5.39	0.07	7.97	0.59	8.73	0.37	8.88	0.65	15.92	1.01
	Refrigerator	0.00	0.00	1.29	1.11	4.14	0.49	6.11	0.44	8.25	0.21	13.52	0.64
Ground water													
HMX	Room temp.	47.53	1.86	41.67	0.57	41.47	2.53	50.63	0.40	45.50	0.35	38.57	1.33
	Refrigerator	47.53	1.86	39.40	2.11	43.73	1.04	47.97	0.47	44.50	2.49	39.57	1.62
RDX	Room temp.	73.17	2.13	66.10	1.11	66.91	0.79	66.47	0.27	68.30	3.29	65.05	1.60
	Refrigerator	73.17	2.13	64.93	1.08	67.22	0.32	69.26	0.96	69.50	2.93	66.03	3.28
TNB	Room temp.	48.97	0.62	47.88	0.26	47.59	0.21	44.02	1.50	42.27	2.11	21.64	2.09
	Refrigerator	48.97	0.62	49.23	0.58	50.28	0.15	49.34	0.32	47.94	0.29	45.36	3.00
TNT	Room temp.	51.06	0.63	50.47	0.42	49.40	0.02	46.88	0.54	41.88	1.90	26.96	1.83
	Refrigerator	51.06	0.63	50.67	0.72	52.52	0.39	50.66	0.40	48.74	0.54	46.17	3.66
2,4-DNT	Room temp.	41.50	1.22	40.82	0.98	41.81	0.38	41.80	0.93	42.24	0.32	37.94	1.12
	Refrigerator	41.50	1.22	40.90	1.04	42.05	0.61	41.91	0.28	41.10	0.95	39.13	2.41
4-AmDNT	Room temp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Refrigerator	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,5-DNA	Room temp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.92	1.53
	Refrigerator	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93	0.83

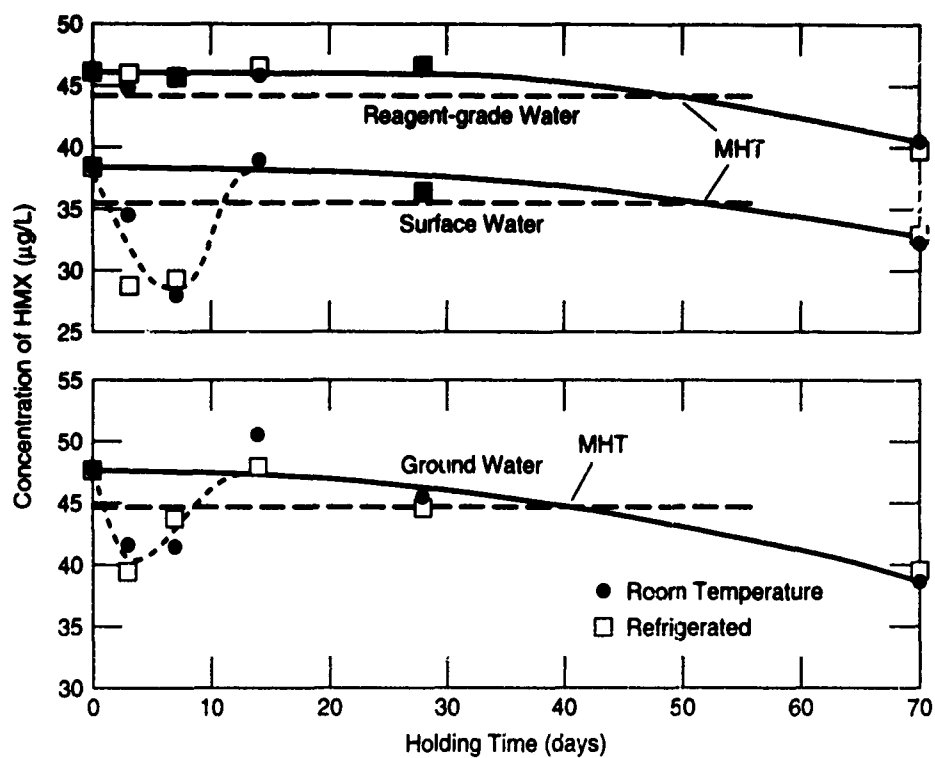


Figure 4. Effect of holding time on HMX concentrations in three water samples. The horizontal dashed lines represent the lower 99% confidence limit of the day 0 means.

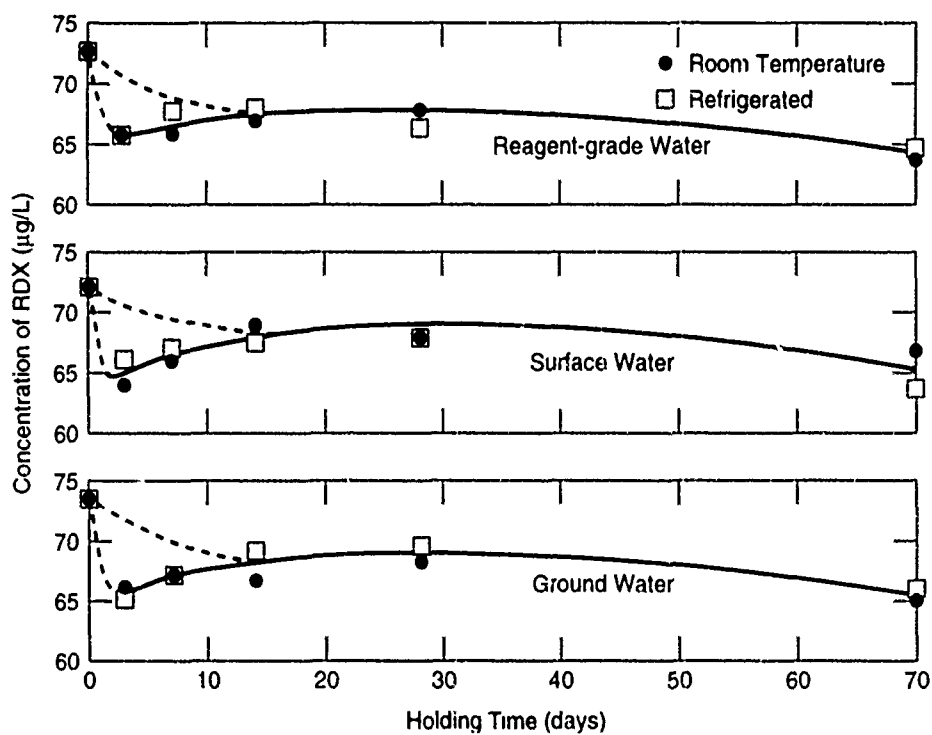


Figure 5. Effect of holding time on RDX concentrations in three water samples.

reality the mean decrease in concentration from day 0 to day 70 is only 10.3%! Given this very small and consistent decrease in concentration for all three water samples at both storage temperatures, we believe that an MHT of 50 days is reasonable. (The mean decrease in concentration at 50 days is only about 6.8%.) Such a result is in agreement with Maskarinec et al. (1991) and Harvey et al. (1991) and is consistent with results from Hoffsommer et al. (1978) and Spanggord et al. (1980), who showed that RDX does not biodegrade under aerobic conditions. It is also in agreement with our earlier findings for soil MHTs (Grant et al. 1993).

### Nitroaromatics

The most stable compound among the nitroaromatics was 2,4-DNT (Fig. 6). As with the nitramines, there was no significant difference between the two storage temperatures. However, the rate of degradation in the surface water was greater than for the reagent-grade water and ground water, and that led to an MHT estimate of 30 days for the former compared to 60 days for the other two. Still, the loss of 2,4-DNT in surface water after 30 days was only 3.6%. If the MHT was arbitrarily increased to 50 days, the loss would still only be 8.3% for the surface water. Since 15% has been specified by the ASTM as an acceptable limit,

a value of 8.3% does not seem excessive.

TNB and TNT present a drastically different picture from the other analytes. In all cases the loss is significantly greater when waters are stored at room temperature than when stored refrigerated. Consequently we will focus on the data from refrigerated samples since it is desirable to stabilize samples as much as possible. Figures 7 and 8 show that both TNB and TNT are quite stable in reagent-grade water and ground water samples, which are represented on each figure by a single line. Estimated MHTs for these refrigerated samples are 63 days for TNB and 60 days for TNT. However, in surface water, both TNB and TNT degrade rapidly, with the accompanying appearance of one of their microbiological degradation products, 3,5-DNA and 4-AmDNT, respectively. After seven days, only 45% of the TNB remains, compared to 70% for TNT. The degradation product of TNB, 3,5-DNA, appears in the day 3 sample, whereas 4-AmDNT, the degradation product of TNT, first appears in the day 14 sample.

### ADDITIONAL COMMENTS

For relatively clean water (i.e. aquatic samples with low microbiological activity), it appears that an MHT of 50 days using refrigeration at 2°C

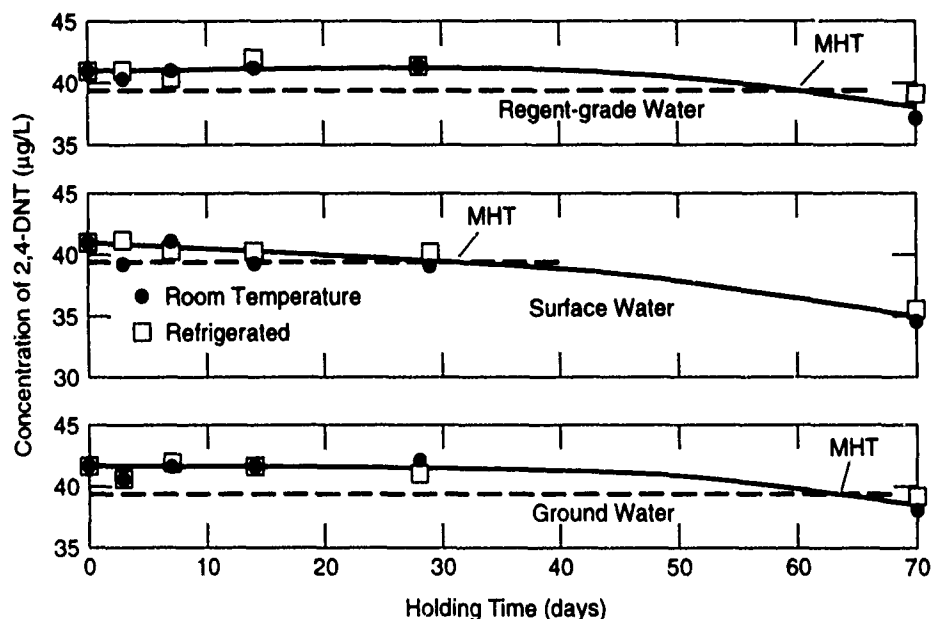


Figure 6. Effect of holding time on 2,4-DNT concentrations in three water samples. The horizontal dashed lines represent the lower 99% confidence limit of the day 0 means.

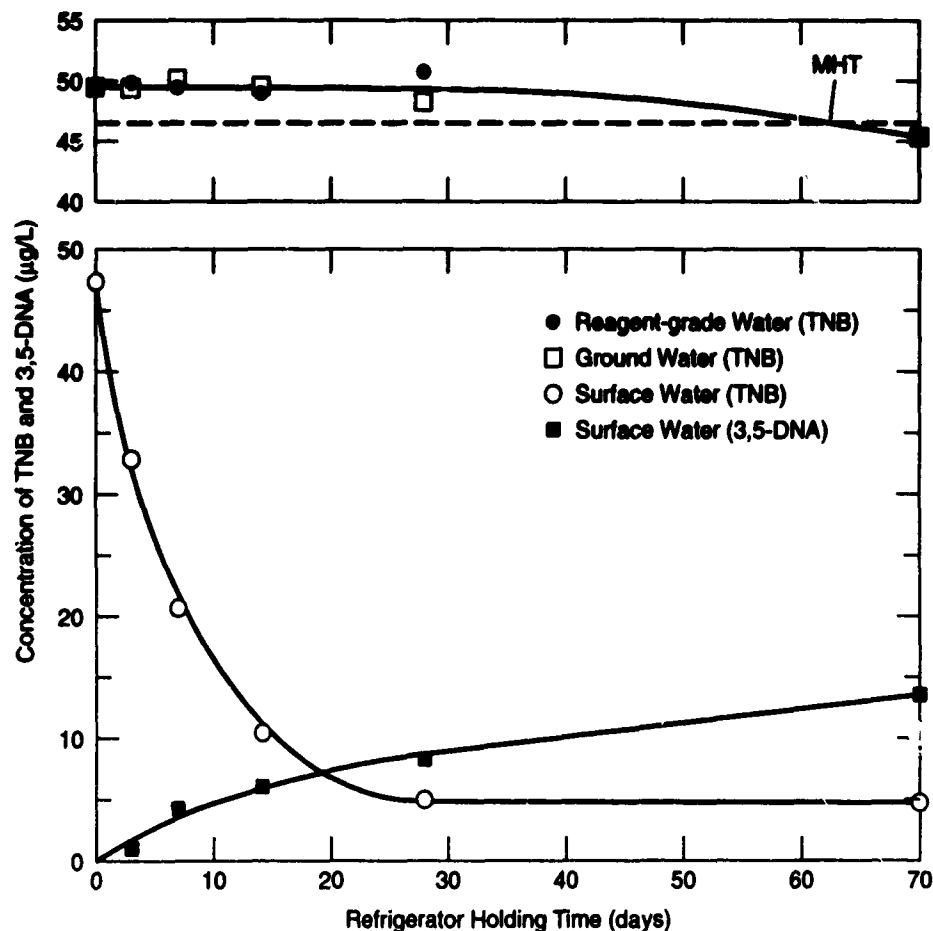


Figure 7. Effect of refrigerator holding time on TNB and 3,5-DNA concentrations in three water samples. The horizontal dashed lines represent the lower 99% confidence limit of the day 0 means.

would be acceptable for both nitramines and nitroaromatics. However, surface water samples, which normally have substantially larger microbial activity, total organic carbon and solids content, will lose significant TNB and TNT within a day or two, even under refrigeration. In a previous study of soils (Grant et al. 1993), we reported that fortified TNB and TNT degraded much more rapidly than the same compounds in field-contaminated soils. This observation remains to be investigated for water samples. Meanwhile, we believe that easily degraded compounds like TNB and TNT in surface water samples should either be preserved by adding appropriate chemicals (as yet undetermined) during sample collection or be analyzed on site with field-portable or on-site methods. Any water samples containing HMX, RDX and 2,4-DNT can be refrigerated for up to 50

days, and relatively pure water samples containing TNB and TNT can also be similarly stored.

At the outset of this study, we hypothesized that the addition of ACN during analyte fortification of water might modify microbial activity and consequently the stability of munitions residues. Comparison of our results (no ACN) with those of Maskarinec et al. (1991) indicates that the low concentration of ACN in the latter study had no detectable effect on MHT estimates for HMX and RDX, which were stable under all conditions tested. For low concentrations of TNT and 2,4-DNT, Maskarinec et al. reported rapid degradation in all waters tested, even under refrigeration. In contrast, we found 2,4-DNT to be very stable in all waters tested, and TNT was stable in refrigerated reagent-grade water and ground water. However, TNT degraded rapidly in refrigerated surface



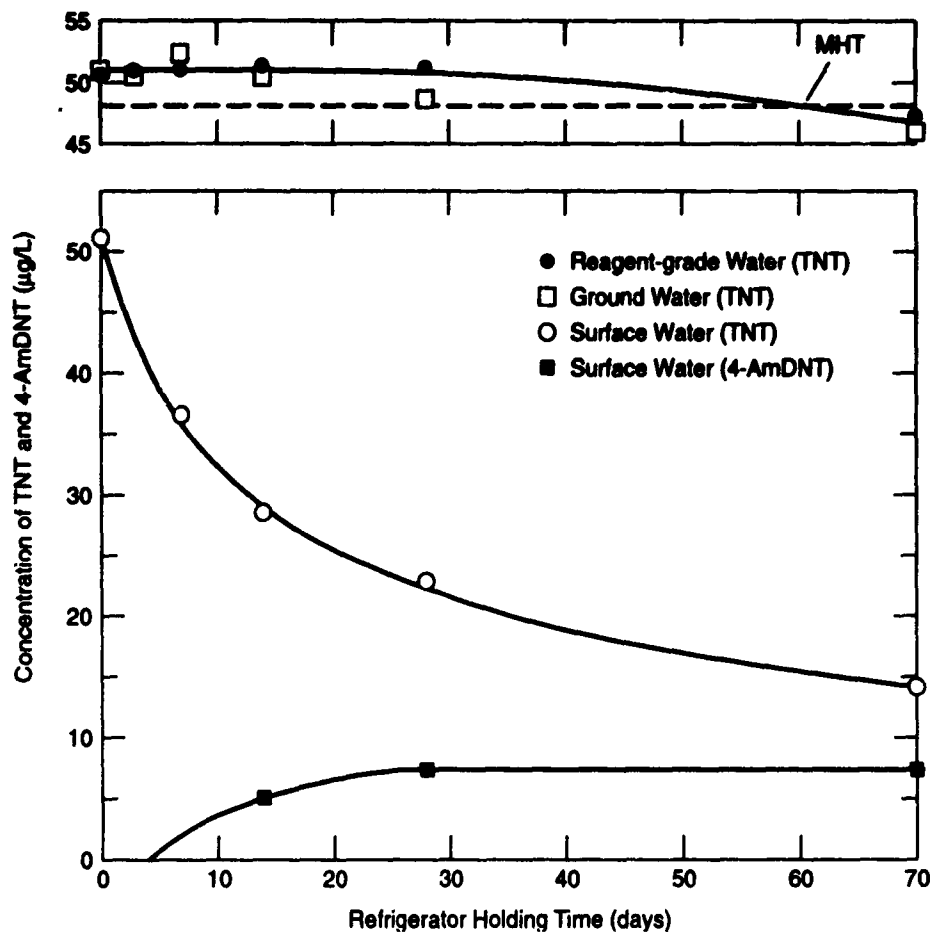


Figure 8. Effect of refrigerator holding time on TNT and 4-AmDNT concentrations in three water samples. The horizontal dashed lines represent the lower 99% confidence limit of the day 0 means.

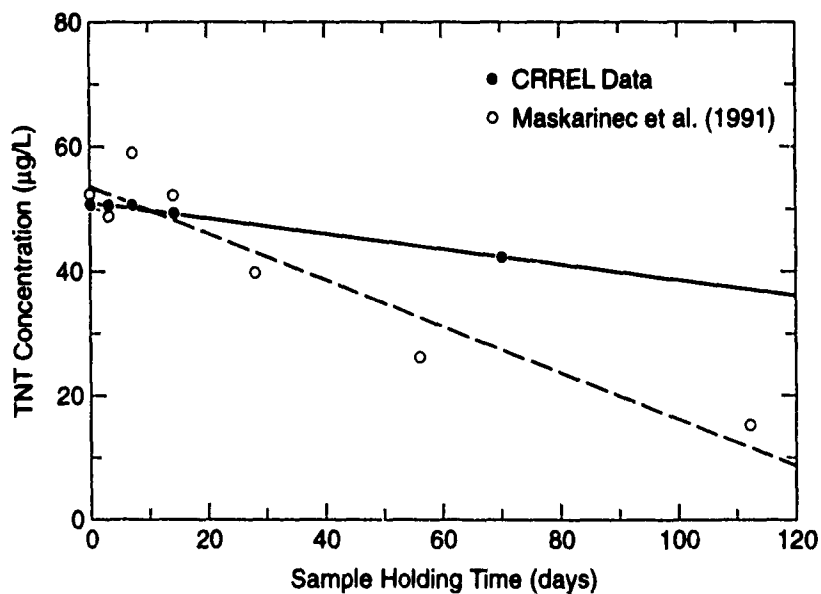


Figure 9. Comparison of TNT stability with time for reagent-grade water in the CRREL and Maskarinec et al. (1991) studies.

water. Maskarinec et al. did not study TNB, so no comparison is possible. Since the ground and surface waters were different in the two studies, we cannot be certain whether the observed differences were due to the presence or absence of ACN or to other compositional differences between the waters. The large differences observed in the stability of these compounds in reagent waters (Fig. 9), however, does strengthen our view that the presence of ACN is a major factor. In any case, we believe that the use of organic solvents should be avoided during analyte fortification of aqueous-based systems that are to be held for any extended period of time prior to analysis. We also believe that this issue deserves immediate further study because of the common practice of using organic solvents during analyte fortification of natural systems.

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# REPORT DOCUMENTATION PAGE

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13. ABSTRACT (Maximum 200 words) <p>A study was conducted to experimentally evaluate the maximum acceptable pre-extraction analytical holding times (MHTs) for three nitroaromatic compounds and two nitramines in water. Three fortified waters (reagent-grade water, ground water and surface water) were utilized in the study. Analytes investigated were HMX, RDX, TNB, TNT and 2,4-DNT, all at the low <math>\mu\text{g/L}</math> level. Subsamples of each water sample were held for periods of 0, 3, 7, 14, 28 and 70 days at either room temperature (22°C) or under refrigeration (2°C). Samples were analyzed by RP-HPLC without preconcentration.</p> <p>The two nitramines, HMX and RDX, were stable over the entire period for all waters under both storage temperatures. For the three nitroaromatics (TNB, TNT and 2,4-DNT) the results were very different. For the surface water, significant losses of TNB and TNT occurred in only a day or two, even under refrigeration. Loss of these analytes was accompanied by an increase in the concentration of their microbiological degradation products 3,5-dinitroaniline and 4-amino-2,6-dinitrotoluene, respectively. The rate of loss of 2,4-DNT was much slower in the surface water than for either TNB or TNT. The rates of loss of the three nitroaromatics were much slower in the reagent-grade water and ground water than in the surface water. The recommended MHT for nitroaromatics and nitramines in relatively sterile water samples is 50 days under refrigeration. Surface water samples, or samples likely to have significant microbial activity, should be preserved in the field prior to shipment or analyzed on-site, or the loss of TNB and TNT is likely to be significant. Concern is voiced over the use of organic solvents for fortification in holding time studies because of their potential for enhancement or suppression of microbiological degradation rates.</p>					
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